STIC-Biotech/ChemLib

53691

From: Ramirez, Delia
Sent: Wednesday, October 24, 2001 4:26 PM
To: STIC-Biotech/ChemLib
Subject: search 09/488265

Hi,

I would like to request the following searches (appl. 09/488265, Lehman et al.)

1. a standard search of seq id 25, 28, 30 in the nucleic acid databases (intereference)
2. a standard search of seq id 26, 27, 29, 31 in the protein databases (intereference)

Thanks,

Delia M. Ramirez, Ph.D.
Patent Examiner - Art Unit 1652 -Mail room 10C01
USPTO
1911 S. Clark Street, Crystal Mall 1, 4E18
Arlington, VA 22202
(703) 306-0288
delia.ramirez@uspto.gov

P

SEARCH REQUEST FORM

Access DB# 53691

Scientific and Technical Information Center

Requester's Full Name: ______ Examiner #: ______ Date: ______

Art Unit: ______ Phone Number 30______ Serial Number: ______

Mail Box and Bldg/Room Location: ______ Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: ______

Inventors (please provide full names): ______

Earliest Priority Filing Date: ______

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

STAFF USE ONLY

STAFF USE ONLY		Type of Search		Vendors and cost where applicable	
Searcher		NA Sequence (#)	3	STN	
Searcher Phone #		AA Sequence (#)	4	Dialog	
Searcher Location		Structure (#)		Questel/Orbit	
Date Searcher Picked Up	10/25/01	Bibliographic		Dr. Link	
Date Completed	10/27/01	Litigation		Lexis/Nexis	
Searcher Prep & Review Time		Fulltext		Sequence Systems	04
Clerical Prep Time		Patent Family		WWW/Internet	
Online Time		Other		Other (specify)	

PTO-1590 (1-2000)

WEST

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Search Results - Record(s) 1 through 3 of 3 returned.

☐ 1. Document ID: US 6153418 A

L3: Entry 1 of 3 File: USPT Nov 28, 2000

US-PAT-NO: 6153418
DOCUMENT-IDENTIFIER: US 6153418 A

TITLE: Consensus phytases

DATE-ISSUED: November 28, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lehmann; Martin	Inzlingen			DEX

US-CL-CURRENT: 435/195; 424/94.1, 424/94.6, 435/196

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw. Desc | Image

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☐ 2. Document ID: EP 897985 A2

L3: Entry 2 of 3 File: EPAB Feb 24, 1999

PUB-NO: EP000897985A2
DOCUMENT-IDENTIFIER: EP 897985 A2
TITLE: Consensus phytases

PUBN-DATE: February 24, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
LEHMANN, MARTIN	DE

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 9/00; A23L 1/03; A23K 1/165; A61K 38/46
EUR-CL (EPC): C12N009/16

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw. Desc | Image

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☐ 3. Document ID: AU 200020928 A, WO 200043503 A1

L3: Entry 3 of 3 File: DWPI Aug 7, 2000

DERWENT-ACC-NO: 2000-491161
DERWENT-WEEK: 200055

TITLE: Novel phytases with improved properties such as temperature stability, pH stability and substrate specificity, for use in pharmaceuticals and compound foods and feeds

INVENTOR: LEHMANN, M

PRIORITY-DATA: 1999DK-0001340 (September 21, 1999), 1999DK-0000092 (January 22, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200020928 A	August 7, 2000		000	C12N009/16
WO 200043503 A1	July 27, 2000	E	238	C12N009/16

INT-CL (IPC): C12N 9/16

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw. Desc | Image

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Terms	Documents
phytase? and lehman?	3

Display 10 Documents, starting with Document: 3

Display Format: CIT Change Format

=> d his

(FILE 'HOME' ENTERED AT 16:30:51 ON 24 OCT 2001)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:31:02 ON
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SEA PHYTAS?

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486 FILE AGRICOLA
12 FILE ANABSTR
33 FILE AQUASCI
346 FILE BIOBUSINESS
32 FILE BIOCOMMERCE
1149 FILE BIOSIS
222 FILE BIOTECHABS
222 FILE BIOTECHDS
170 FILE BIOTECHNO
916 FILE CABA
5 FILE CANCERLIT
1555 FILE CAPLUS
92 FILE CEABA-VTB
3 FILE CEN
19 FILE CIN
67 FILE CONFSCI
6 FILE CROPB
4 FILE CROPU
2 FILE DDFB
4 FILE DDFU
1262 FILE DGENE
2 FILE DRUGB
6 FILE DRUGU
1 FILE DRUGUPDATES
8 FILE EMBAL
235 FILE EMBASE
212 FILE ESBIOBASE
2 FILE FOREGE
247 FILE FROSTI
263 FILE FSTA
302 FILE GENBANK

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          2  FILE HEALSAFE
         99  FILE IFIPAT
        107  FILE JICST-EPLUS
        160  FILE LIFESCI
        411  FILE MEDLINE
          2  FILE NIOSHTIC
          3  FILE NTIS
         11  FILE OCEAN
        380  FILE PASCAL
         66  FILE PHIN
        127  FILE PROMT
        844  FILE SCISEARCH
        129  FILE TOXLIT
        435  FILE USPATFULL
        190  FILE WPIDS
        190  FILE WPINDEX
          3  FILE NAPRALERT
         44  FILE NLDB
L1          QUE PHYTAS?
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   FILE 'CAPLUS, DGENE, BIOSIS, CABA, SCISEARCH, AGRICOLA, USPATFULL,
   MEDLINE, PASCAL, BIOBUSINESS, GENBANK, FSTA, FROSTI, EMBASE,
BIOTECHDS,
   ESBIOBASE, WPIDS, BIOTECHNO, LIFESCI, TOXLIT, PROMT, JICST-EPLUS, IFIPAT,
   CEABA-VTB, CONFSCI, PHIN, NLDB, AQUASCI, ...' ENTERED AT 16:31:50 ON 24
   OCT 2001
L2       10623 S PHYTAS?
L3        5507 DUP REM L2 (5116 DUPLICATES REMOVED)
L4        5507 S L3 AND PHYTAS?
L5           0 S L4 AND LEHMA?
L6           0 S L4 AND LEHMAN?
L7         178 S L4 AND MARTIN?

=> s l4 and lassen?
L8           1 L4 AND LASSEN?
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 NEWS  2  Dec 17  The CA Lexicon available in the CAPLUS and CA files
 NEWS  3  Feb 06  Engineering Information Encompass files have new names
 NEWS  4  Feb 16  TOXLINE no longer being updated
 NEWS  5  Apr 23  Search Derwent WPINDEX by chemical structure
 NEWS  6  Apr 23  PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
 NEWS  7  May 07  DGENE Reload
 NEWS  8  Jun 20  Published patent applications (A1) are now in USPATFULL
 NEWS  9  JUL 13  New SDI alert frequency now available in Derwent's
                  DWPI and DPCI
 NEWS 10  Aug 23  In-process records and more frequent updates now in
                  MEDLINE
 NEWS 11  Aug 23  PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA
 NEWS 12  Aug 23  Adis Newsletters (ADISNEWS) now available on STN
 NEWS 13  Sep 17  IMSworld Pharmaceutical Company Directory name change
                  to PHARMASEARCH
 NEWS 14  Oct 09  Korean abstracts now included in Derwent World Patents
                  Index
 NEWS 15  Oct 09  Number of Derwent World Patents Index updates increased
 NEWS 16  Oct 15  Calculated properties now in the REGISTRY/ZREGISTRY File
 NEWS 17  Oct 22  Over 1 million reactions added to CASREACT
 NEWS 18  Oct 22  DGENE GETSIM has been improved

 NEWS EXPRESS  August 15 CURRENT WINDOWS VERSION IS V6.0c,
               CURRENT MACINTOSH VERSION IS V6.0 (ENG) AND V6.0J (JP),
               AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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=> index bioscience medicine
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                SINCE FILE     TOTAL
                                                         ENTRY   SESSION
FULL ESTIMATED COST                                       0.15      0.15
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INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
       BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
       CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
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62 FILES IN THE FILE LIST IN STNINDEX

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=> s phytas?
        486   FILE AGRICOLA
         12   FILE ANABSTR
         33   FILE AQUASCI
        346   FILE BIOBUSINESS
         32   FILE BIOCOMMERCE
       1149   FILE BIOSIS
        222   FILE BIOTECHABS
        222   FILE BIOTECHDS
        170   FILE BIOTECHNO
        916   FILE CABA
          5   FILE CANCERLIT
       1555   FILE CAPLUS
         92   FILE CEABA-VTB
          3   FILE CEN
         19   FILE CIN
         67   FILE CONFSCI
          6   FILE CROPB
          4   FILE CROPU
          2   FILE DDFB
          4   FILE DDFU
       1262   FILE DGENE
          2   FILE DRUGB
          6   FILE DRUGU
          1   FILE DRUGUPDATES
          8   FILE EMBAL
        235   FILE EMBASE
        212   FILE ESBIOBASE
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        247   FILE FROSTI
        263   FILE FSTA
        302   FILE GENBANK
          2   FILE HEALSAFE
         99   FILE IFIPAT
        107   FILE JICST-EPLUS
  42 FILES SEARCHED...
        160   FILE LIFESCI
        411   FILE MEDLINE
          2   FILE NIOSHTIC
          3   FILE NTIS
         11   FILE OCEAN
        380   FILE PASCAL
         66   FILE PHIN
        127   FILE PROMT
        844   FILE SCISEARCH
        129   FILE TOXLIT
        435   FILE USPATFULL
        190   FILE WPIDS
        190   FILE WPINDEX
          3   FILE NAPRALERT
         44   FILE NLDB
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49 FILES HAVE ONE OR MORE ANSWERS, 62 FILES SEARCHED IN STNINDEX

L1 QUE PHYTAS?

=> d rank

F1	1555	CAPLUS
F2	1262	DGENE
F3	1149	BIOSIS
F4	916	CABA
F5	844	SCISEARCH
F6	486	AGRICOLA
F7	435	USPATFULL
F8	411	MEDLINE
F9	380	PASCAL
F10	346	BIOBUSINESS
F11	302	GENBANK
F12	263	FSTA
F13	247	FROSTI
F14	235	EMBASE
F15	222	BIOTECHABS
F16	222	BIOTECHDS
F17	212	ESBIOBASE
F18	190	WPIDS
F19	190	WPINDEX
F20	170	BIOTECHNO
F21	160	LIFESCI
F22	129	TOXLIT
F23	127	PROMT
F24	107	JICST-EPLUS
F25	99	IFIPAT
F26	92	CEABA-VTB
F27	67	CONFSCI
F28	66	PHIN
F29	44	NLDB
F30	33	AQUASCI
F31	32	BIOCOMMERCE
F32	19	CIN
F33	12	ANABSTR
F34	11	OCEAN
F35	8	EMBAL
F36	6	CROPB
F37	6	DRUGU
F38	5	CANCERLIT
F39	4	CROPU
F40	4	DDFU
F41	3	CEN
F42	3	NTIS
F43	3	NAPRALERT
F44	2	DDFB
F45	2	DRUGB
F46	2	FOREGE
F47	2	HEALSAFE
F48	2	NIOSHTIC
F49	1	DRUGUPDATES

=> file f1-f34

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=> s phytas?
L2       10623 PHYTAS?

=> dup rem l2
DUPLICATE IS NOT AVAILABLE IN 'DGENE, GENBANK, BIOCOMMERCE'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING IS APPROXIMATELY   9% COMPLETE FOR L2
PROCESSING IS APPROXIMATELY  37% COMPLETE FOR L2
PROCESSING IS APPROXIMATELY  53% COMPLETE FOR L2
PROCESSING IS APPROXIMATELY  66% COMPLETE FOR L2
PROCESSING IS APPROXIMATELY  78% COMPLETE FOR L2
PROCESSING IS APPROXIMATELY  87% COMPLETE FOR L2
PROCESSING IS APPROXIMATELY  95% COMPLETE FOR L2
PROCESSING COMPLETED FOR L2
L3          5507 DUP REM L2 (5116 DUPLICATES REMOVED)

=> s l3 and phytas?
   4 FILES SEARCHED...
  14 FILES SEARCHED...
  24 FILES SEARCHED...
L4         5507 L3 AND PHYTAS?
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=> s l4 and lehma?
L5            0 L4 AND LEHMA?

=> s l4 and lehman?
L6            0 L4 AND LEHMAN?

=> s l4 and martin?
L7          178 L4 AND MARTIN?

=> d ti 1-178
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STNGUIDE file for information on formats available in individual
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REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):l7
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     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:31:02 ON
     24 OCT 2001
                SEA PHYTAS?
               ---------
             486   FILE AGRICOLA
              12   FILE ANABSTR
              33   FILE AQUASCI
             346   FILE BIOBUSINESS
              32   FILE BIOCOMMERCE
            1149   FILE BIOSIS
             222   FILE BIOTECHABS
             222   FILE BIOTECHDS
             170   FILE BIOTECHNO
             916   FILE CABA
               5   FILE CANCERLIT
            1555   FILE CAPLUS
              92   FILE CEABA-VTB
               3   FILE CEN
              19   FILE CIN
              67   FILE CONFSCI
               6   FILE CROPB
               4   FILE CROPU
               2   FILE DDFB
               4   FILE DDFU
            1262   FILE DGENE
               2   FILE DRUGB
               6   FILE DRUGU
               1   FILE DRUGUPDATES
               8   FILE EMBAL
             235   FILE EMBASE
             212   FILE ESBIOBASE
               2   FILE FOREGE
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              247   FILE FROSTI
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              302   FILE GENBANK
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              107   FILE JICST-EPLUS
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              411   FILE MEDLINE
                2   FILE NIOSHTIC
                3   FILE NTIS
               11   FILE OCEAN
              380   FILE PASCAL
               66   FILE PHIN
              127   FILE PROMT
              844   FILE SCISEARCH
              129   FILE TOXLIT
              435   FILE USPATFULL
              190   FILE WPIDS
              190   FILE WPINDEX
                3   FILE NAPRALERT
               44   FILE NLDB
L1               QUE PHYTAS?
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     FILE 'CAPLUS, DGENE, BIOSIS, CABA, SCISEARCH, AGRICOLA, USPATFULL,
     MEDLINE, PASCAL, BIOBUSINESS, GENBANK, FSTA, FROSTI, EMBASE, BIOTECHDS,
     ESBIOBASE, WPIDS, BIOTECHNO, LIFESCI, TOXLIT, PROMT, JICST-EPLUS, IFIPAT,
     CEABA-VTB, CONFSCI, PHIN, NLDB, AQUASCI, ...' ENTERED AT 16:31:50 ON 24
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L2          10623 S PHYTAS?
L3           5507 DUP REM L2 (5116 DUPLICATES REMOVED)
L4           5507 S L3 AND PHYTAS?
L5              0 S L4 AND LEHMA?
L6              0 S L4 AND LEHMAN?
L7            178 S L4 AND MARTIN?

=> d l7 1-178 ti
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=> d 1-10

L7   ANSWER 1 OF 178  USPATFULL
AN     2001:185559  USPATFULL
TI     Hybrid maize plant & seed 34T38
IN     Whitaker, David Walter, Narvon, PA, United States
PA     Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.
       corporation)
PI     US 6307132          B1    20011023
AI     US 1999-376036             19990817 (9)
DT     Utility
FS     GRANTED
LN.CNT 1664
INCL   INCLM: 800/320.100
       INCLS: 800/298.000; 800/275.000; 800/271.000; 800/268.000; 800/266.000;
              800/301.000; 800/302.000; 800/303.000; 435/412.000; 435/424.000;
              435/430.000; 435/430.100
NCL    NCLM:  800/320.100
       NCLS:  800/298.000; 800/275.000; 800/271.000; 800/268.000; 800/266.000;
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800/301.000; 800/302.000; 800/303.000; 435/412.000; 435/424.000;
435/430.000; 435/430.100

IC [7]
ICM: A01H005-00
ICS: A01H004-00; A01H001-00; C12N005-04
EXF 800/320.1; 800/298; 800/275; 800/271; 800/268; 800/266; 435/412;
435/422; 435/430; 435/430.1

L7 ANSWER 2 OF 178 USPATFULL
AN 2001:185558 USPATFULL
TI Soybean variety 93B66
IN Corbin, Thomas C., Monticello, IL, United States
Freestone, Robert E., Cedar Falls, IA, United States
PA Pioneer Hi-Bred International, Inc., United States (U.S. corporation)
PI US 6307131 B1 20011023
AI US 2000-500903 20000209 (9)
DT Utility
FS GRANTED
LN.CNT 1456
INCL INCLM: 800/312.000
INCLS: 800/260.000; 800/265.000; 800/266.000; 800/267.000; 800/268.000;
800/278.000; 800/300.000; 435/415.000; 435/419.000; 435/421.000;
435/426.000; 435/430.000; 435/430.100; 435/468.000
NCL NCLM: 800/312.000
NCLS: 800/260.000; 800/265.000; 800/266.000; 800/267.000; 800/268.000;
800/278.000; 800/300.000; 435/415.000; 435/419.000; 435/421.000;
435/426.000; 435/430.000; 435/430.100; 435/468.000
IC [7]
ICM: A01H001-04
ICS: A01H005-00; A01H005-10; A01H004-00; C12N005-04
EXF 800/260; 800/265; 800/266; 800/267; 800/268; 800/278; 800/300; 800/312;
435/415; 435/419; 435/421; 435/426; 435/430; 435/430.1; 435/468

L7 ANSWER 3 OF 178 USPATFULL
AN 2001:185550 USPATFULL
TI Methods and compositions for transgene identification
IN Kriz, Alan L., Gales Ferry, CT, United States
Spencer, T. Michael, Mystic, CT, United States
PA Dekalb Genetics Corporation, Dekalb, IL, United States (U.S.
corporation)
PI US 6307123 B1 20011023
AI US 1998-80625 19980518 (9)
DT Utility
FS GRANTED
LN.CNT 5135
INCL INCLM: 800/282.000
INCLS: 800/266.000; 800/288.000; 800/300.000; 800/301.000; 536/023.400;
536/024.100
NCL NCLM: 800/282.000
NCLS: 800/266.000; 800/288.000; 800/300.000; 800/301.000; 536/023.400;
536/024.100
IC [7]
ICM: C12N015-00
ICS: C12M015-09; A01H005-00; A01H001-02
EXF 536/24.1; 536/23.4; 435/69.1; 435/172.3; 435/410; 435/411; 435/419;
435/430.1; 800/278; 800/266; 800/282; 800/288; 800/200; 800/301
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 4 OF 178 USPATFULL
AN 2001:179335 USPATFULL
TI Hybrid maize plant & seed 39n03
IN Kramer, Joachim Ernst, Neusiedl, Austria
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.

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        corporation)
PI      US 6303851          B1    20011016
AI      US 2000-519974            20000307 (9)
RLI     Continuation of Ser. No. US 1999-258554, filed on 26 Feb 1999, now
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DT      Utility
FS      GRANTED
LN.CNT 1603
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        435/412; 435/424; 435/430; 435/430.1

L7   ANSWER 5 OF 178  USPATFULL
AN      2001:170893  USPATFULL
TI      Methods and compositions for the introduction of molecules into cells
IN      Held, Bruce Marvin, Ames, IA, United States
        Wilson, Herbert Martin, Ames, IA, United States
        Hou, Liming, St. Louis, MO, United States
        Lewnau, Carol Jean, Ames, IA, United States
        Eby, Janelle Christine, Ames, IA, United States
PI      US 2001026941       A1    20011004
AI      US 2001-850476      A1    20010507 (9)
RLI     Continuation of Ser. No. US 1999-450226, filed on 29 Nov 1999, PENDING
DT      Utility
FS      APPLICATION
LN.CNT 1907
INCL    INCLM: 435/468.000
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L7   ANSWER 6 OF 178  USPATFULL
AN      2001:168312  USPATFULL
TI      Hybrid maize plant and seed 35H53
IN      Chapman, Michael A., Madison Lake, MN, United States
PA      Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.
        corporation)
PI      US 6297433          B1    20011002
AI      US 2000-489136            20000121 (9)
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EXF   800/320.1; 800/298; 800/275; 800/271; 800/268; 800/301; 800/302;
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L7   ANSWER 7 OF 178 USPATFULL
AN   2001:168311 USPATFULL
TI   Hybrid maize plant and seed 39A26
IN   Puskaric, Vladimir, Woodstock, Canada
PA   Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.
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PI   US 6297432   B1   20011002
AI   US 1999-259868   19990301 (9)
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EXF   800/320.1; 800/298; 800/278; 800/271; 800/268; 800/266; 435/412;
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L7   ANSWER 8 OF 178 USPATFULL
AN   2001:158041 USPATFULL
TI   Alkaline protease deficient filamentaous fungi
IN   Lehmbeck, Jan, Veks.o slashed., Denmark
PA   Novo Nordisk A/S Novo alle, Bagsvaerd, Denmark (non-U.S. corporation)
PI   US 6291209   B1   20010918
AI   US 1998-148751   19980904 (9)
RLI   Continuation of Ser. No. WO 1997-DK135, filed on 26 Mar 1997
PRAI   DK 1996-354   19960327
        DK 1996-555   19960509
DT   Utility
FS   GRANTED
LN.CNT 644
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        NCLS: 435/484.000; 435/254.300; 435/320.100
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7   ANSWER 9 OF 178 USPATFULL
AN   2001:153197 USPATFULL
TI   Hybrid maize plant and seed 36D14
IN   Gogerty, Joseph Kevin, Algona, IA, United States
PA   Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.
        corporation)
PI   US 6288310   B1   20010911
AI   US 1999-259989   19990301 (9)
DT   Utility
FS   GRANTED
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IC      [7]
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        ICS: A01H004-00; A01H001-00; C12H005-04
EXF     800/320.1; 800/298; 800/275; 800/271; 800/301-303; 435/412; 435/424;
        435/430; 435/430.1

L7    ANSWER 10 OF 178  USPATFULL
AN      2001:148156  USPATFULL
TI      Hybrid maize plant and seed 32Y52
IN      Colbert, Terry R., Fort Branch, IN, United States
PA      Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.
        corporation)
PI      US 6284953          B1    20010904
AI      US 1999-237518            19990126 (9)
DT      Utility
FS      GRANTED
LN.CNT 1871
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L7            178 S L4 AND MARTIN?

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L8              1 L4 AND LASSEN?

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L8   ANSWER 1 OF 1  PROMT  COPYRIGHT 2001 Gale Group

ACCESSION NUMBER:   2001:356673  PROMT
TITLE:              New strategies in supplementation of phosphorus discussed.
AUTHOR(S):          BRUYNE, KARL DE
SOURCE:             Feedstuffs, (6 Mar 2000) Vol. 72, No. 10, pp. 11.
                    ISSN: 0014-9624.
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LANGUAGE:           English
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L8 ANSWER 1 OF 1 PROMT

AN 2001:356673 PROMT
TI New strategies in supplementation of phosphorus discussed.
AU BRUYNE, KARL DE
SO Feedstuffs, (6 Mar 2000) Vol. 72, No. 10, pp. 11.
ISSN: 0014-9624.
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TX ABSTRACT

This paper presents several aspects of the use of **phytase** in swine and poultry diets, including the effect of **phytase** on other minerals, amino acids, water consumption and stability. Considerations for the inclusion of modern feed phosphates are also discussed.

Phytase increases digestibility of vegetal phosphorus and calcium. It is well documented that **phytase** increases digestibility of both phosphorus and calcium. Dutch research established in the beginning of the 1990s (Jongbloed, 1993) a **phytase** activity equivalence to feed phosphates of 500 IU **phytase** = 1 g phosphorus from monocalcium phosphate or 1.1 g phosphorus from dicalcium phosphate dihydrate for pigs. At higher doses, efficacy decreases. This equivalency has been taken over by most **phytase** producers in their recommendations. It is based on a digestibility coefficient for monocalcium phosphate of 80%.

Eeckhout (1997), however, found a digestibility for monocalcium phosphate of 93%. It is also assumed that this equivalency is valid independent of the raw material used. However, literature indicates clearly that the improvement of phosphorus digestibility by **phytase** varies clearly in function of the raw material.

It is most probable that the amount and type of phytic phosphorus and the presence of vegetal **phytase** influences the performance of the microbial **phytase**; it is best when a lot of phytic phosphorus is present (Rodehutscord and Dungelhoef, 1994). Using microbial **phytase**, they improved phosphorus digestibility of corn from 18 to 56%. The phosphorus digestibility of wheat improved from 62 to 74%, while improvement in triticale was from 52 to 67%. The improvement in digestibility is much higher in corn. This indicates that the presence of vegetal **phytase** hydrolyses phytic phosphorus and eliminates substrate for the microbial **phytase**, thus reducing its efficacy.

Another explanation may be the solubility of corn phytate is higher than the solubility of wheat phytate at pH 4-7 (Scheuermann, 1988). Therefore, it is very improbable that the Dutch value, obtained mainly on **phytase**-poor raw materials, is applicable everywhere. It is clear that in countries where a lot of cereals (which contain endogenous **phytase**) are used, the above mentioned equivalency is not valid but should be 700 IU **phytase** = 1 g phosphorus from monocalcium phosphate. In non-pelleted, cereal-rich compound feeds, it is not economical to use microbial **phytase**.

Kemme (1997) came to the conclusion that this equivalency was not valid for every kind of pig and also varied with the age of the pig. Indeed, for

piglets, 500 IU of **phytase** liberate only 0.66 g of digestible phosphorus. He even measured differences in sows at the beginning or end of pregnancy, finding 0.32 and 0.74 g of digestible phosphorus for 500 IU of **phytase**, respectively. This is another factor of variability which complicates formulation with **phytase**.

Phytase increases digestibility of some amino acids. Several researchers have reported that **phytase** improves ileal digestibility of amino acids and nitrogen both in pigs and poultry (Oficer and Batterham, 1992; Mroz et al., 1994; Yi et al., 1994; Farrell et al., 1993; Van de Klis and Versteegh, 1991). In turkeys, Yi et al. (1996) found an improved ileal digestibility for some amino acids, but not for cystine and methionine. Sebastian (1997) also found only a partial effect on amino acid digestibility in male and female broilers. Pallauf (1994) and Newkirk and Classen (1995) found no significant effect on crude protein digestibility. In spite of this possible increased amino acid digestibility, a significant positive effect on feed conversion ratio is seldom observed (Simons et al., 1990; Broz et al., 1994; Denbow et al., 1995; Sebastian, 1996). The possible effect of **phytase** on growth parameters needs to be confirmed. Application at this stage of this "protein effect" could lead to adverse effects. It would be useful to examine the factors causing the variability of the findings.

Phytase activity depends on calcium:phosphorus ratio. Excess dietary calcium has a negative effect on the utilization of phosphorus because of formation of insoluble calcium-phytate complexes (Nelson and Kirby, 1987). Since **phytase** liberates calcium, and thus increases the total amount of available calcium in the feed, it is clear that possible interactions between **phytase** and calcium content have to be monitored. It is also possible that high calcium levels increase pH to levels that are not optimal for **phytase** activity. Liu et al. (1997) found lowering the calcium:phosphorus ratio from 1.5:1.0 to 1.0:1.0 in pigs increased most investigated parameters, except bone breaking strength. Lantzsch et al. (1995) found maximum phosphorus retention at a calcium:phosphorus ratio of 1.5:1.0. Quian et al. (1997) found 1.25:1.0 as optimal ratio. In poultry, some authors (Sebastian, 1996; Schoner, 1993) found improved growth and mineral utilization at lower calcium levels. Huyghebaert (1996) found a higher incidence of t ibial dyschondroplasia (TD) at narrow calcium:phosphorus ratios. It seems that the existing calcium recommendations have to be evaluated in view of **phytase** utilization.

Phytase increases water consumption and causes wet manure. For broilers, it has been proven (Schutte, 1993) that the replacement of 0.5% monocalcium phosphate by 500 IU of microbial **phytase** results in significant increase of water intake and the water:feed ratio 4-5%. There are indications that this is caused by an increase of magnesium and calcium availability, and perhaps also of sodium and potassium. These are all minerals that highly increase the water metabolism and the degree of moisture in excreta. This indirect negative aspect of the application of microbial **phytase** needs to be taken into consideration if one wants to prevent problems with wet droppings and litter.

Phytase gives poorer bone mineralization. As to bone mineralization in poultry, it seems difficult to obtain maximum bone strength or ash content by supplementation of microbial **phytase**, even in doses of 1,000 IU/kg (Sauveur, 1993; Simons, 1993; Cromwell, 1995; Cantor, 1996; Liu et al., 1997) in comparison with the control diet (0.73% phosphorus, 0.45% available phosphorus; Huyghebaert, 1992). This is in spite of the fact that the phosphorus liberated by **phytase** seems to be utilized as efficiently for skeleton mineralization as the added phosphorus from dicalcium phosphate dihydrate. This is because the phosphorus available per animal does not always reach that of the control

diet with exclusively mineral phosphorus supplementation (Simons, 1993; Huyghebaert, 1992).

Phytase activity is influenced by premix ingredients. Practical experience in Holland showed interference between some premix ingredients and microbial **phytase**. Notably, liquid choline and iron sulfate heptahydrate caused important loss of activity after storage. It is mainly the presence of moisture that causes the interaction with the enzyme. It is very probable that minerals with an important content of molasses will also interact with the microbial **phytase**, which will lead to activity loss. This seems to make the use of **phytase** in mineral feed impossible.

Phytase is not thermo-stable. Eeckhout (1998) performed an extensive trial on the influence of steam pelleting and storage temperature on the recovery of **phytase**.

In a first experiment, the influence of hole diameter, channel length and feed composition on the **phytase** activity was measured. It is clearly indicated that all friction-enhancing factors have a negative influence on **phytase** stability (Figures 1,2 and 3). It appears that a die hole of 2 mm causes much more **phytase** activity loss than a die of 4mm. A longer channel length of 40 mm seems also to lower the activity of the **phytase**, mainly at lower conditioning temperatures. In this way, the channel length supplies, via friction, the additional temperature necessary to destroy the **phytase** activity. Feed composition also plays an important role on **phytase** stability. High-fat feeds, like broiler feed, go through the die with less friction and less temperature increase, and hence the recovery of activity is much higher than in the fiber-rich pig feeds.

In a second experiment, the influence of storage temperature on **phytase** activity has been measured. Figures 4 and 5 demonstrate that an additional loss of 20-40% **phytase** activity can be suffered after only two days of storage at 40 and 50[degrees]C, respectively.

In a third experiment, liquid **phytase** was used. Application of liquid **phytase** is difficult and requires an important investment. It sometimes can lead to heterogeneous dispersion of the **phytase** over the pellets. Another important factor is loss of activity due to pneumatic manipulations. Indeed, an important fraction of the **phytase** is applied on the tops of the pellets. As a result, a lot of **phytase** ends up in the meal fraction of the feed. This is often recycled and pelleted again. In this way, the sprayed **phytase** is destroyed. Creation of a meal fraction also happens outside the feed mill on the farm. During storage, the loss of activity of the liquid **phytase** was even greater than for the solid **phytase**. This is probably because the sprayed **phytase** is totally on the outside of the pellet. This experiment indicates that extreme attention has to be paid to the loss of **phytase** activity occurring during production and storage. At very high pelleting and storage temperatures, total destruction of **phytase** is not improbable (Figure 6). It is also shown that a simple rule of thumb accounting for 30% activity loss is not adequate since the major influences are raw materials, conditioning temperature and even die diameter and channel length.

In a fourth experiment, a "protected" **phytase** was tested. This type of **phytase** came on the market a few years after the original **phytase** and after the liquid **phytase**. The argument of the producer for this innovation was the inability of the original **phytase** to survive pelleting. In this way, our earlier

results were indirectly confirmed. In this experiment, we did the now common pelleting test with a 2 x 40 mm die (broiler feed) and a 4 x 50 mm die (piglet feed). Later on, these pellets were stored for two weeks at different temperatures.

The **phytase** recovery in the broiler feed shows an important reduction at 71[degrees]C conditioning temperature. At 82[degrees]C the recovery was 40%. For the piglet feed, comparable results were found; the recovery at high conditioning temperature (82[degrees]C) was 60%. The larger size of this pellet may explain a higher recovery (Figures 7 and 8).

Figures 9 and 10 show the results of the storage experiment. At 30[degrees]C the recovery was reduced to 85-80%. At higher storage temperatures, the loss increased up to 30 (at 40[degrees]C), and even to 60% (at 50[degrees]C). When, due to extreme storage conditions, the temperature in the outer parts of a bin increases, the activity loss may be very important.

The overall recovery of the granulated form shows a higher stability (higher recovery) than the classical **phytase**, which was almost totally lost. However, at 80[degrees]C the activity loss still is very important.

Feed phosphates

Environmental pressure and progress in livestock production resulted in the development of a more precise evaluation system. Started in the 1980s, a method was developed in the Netherlands to express the biological value of phosphorus in the feed as apparent digestible phosphorus by measuring the difference in ingested and excreted phosphorus.

In most countries, feed compounders are still expressing the biological value of raw materials as available phosphorus, in which only little differentiation is made. Therefore, it would be worthwhile to translate the outcome of the digestibility trials into these systems, enabling feed compounders to optimize the diets more closely to the needs of the animal. This would result in a reduction in the excretion of phosphorus into the environment without endangering the animal welfare or economic results.

Digestibility trials in pigs. Although the absolute digestibility levels differ between trials, the ranking between the different types of phosphorus is the same (Table 1). Most probably this is caused by differences in protocol and the statistical analysis applied. It is very important that, when carrying out a digestibility trial, the level of vegetal phosphorus in the basal feed is as low as possible. Otherwise, this phosphorus has a negative interaction with the feed phosphates tested, resulting in an underestimation.

For those reasons, Eeckhout and De Paepe (1997) slightly adapted the digestibility procedure. They used a semi-synthetic diet virtually free of phosphorus, 0.037%. There were two treatments per phosphate: 1 or 2 g added phosphorus/kg. Calcium was added at 5 g/kg. Liveweight of the animals was between 35 and 40 kg.

To counteract marker problems and the high variability of phosphorus digestibility, a total collection of feces in metabolic cages was applied over a 10-day period. In order to guarantee the accurate intake of the calculated amounts of phosphorus and calcium, the different feed phosphates and calcium carbonate were carefully weighed, per feeding and per pig, and hand mixed in the individual feeding troughs.

Results indicated the feed phosphates with the highest digestibility

coefficients have been underestimated in earher trials. It is therefore suggested to use to the Eeckhout procedure (1997) for future digestibility trials.

Digestibility trials in poultry. Poultry and pigs are quite different with respect to the anatomy of their digestive system. Therefore, poultry and pigs digest feed phosphates quite differently. The trials mentioned in Table 2 were also done with synthetic vegetal phosphorus-free basal diets.

Between the two trials, the results are quite comparable. The ranking between the different types is more or less the same as found in the trials with pigs, although there are some differences in the absolute digestibility values. In particular, the value for dihydrated dicalcium phosphate is much higher with poultry than with pigs. Poultry seem to digest dihydrated dicalcium phosphate much better. Defluorinated phosphate and anhydrous dicalcium phosphate seem not to be suited for poultry.

Based on the above mentioned research data, we propose in Table 3 a digestibility coefficient for most commercial feed phosphates. These values can be used for evaluating feed phosphates or even for formulating feeds with digestible phosphorus constraints.

As a conclusion, it seems that dihydrated dicalcium phosphate and monocalcium phosphate are particularly suitable for poultry, whereas monocalcium and monodicalcium phosphate are preferred for pigs.

Conclusions

In spite of the fact that European livestock, although rather concentrated in some areas, is not confronted with the same environmental problems in every region, it seems worthwhile to pay some attention to phosphorus supply of the animals. As indicated, two main strategies exist. The first one is using **phytase**. This enzyme offers certainly new perspectives in reducing phosphorus excretion. The main disadvantage of this product is its variability. Its efficacy depends on the raw materials used in the feeds and is affected by production parameters such as premix ingredients, conditioning temperatures, die diameter, channel length, fiber content of the feed and storage temperatures. This makes it impossible for the feed compounder to predict its value and hence to work with **phytase** without using safety margins. One of the most important goals of **phytase**, avoiding excessive excretion of phosphorus in the manure, is in this way difficult to achieve. Since there is no danger for phosphorus saturation of most Euro pean soils, it is not useful to take unnecessary risks. It is certainly not necessary to take these risks since modern high-quality feed phosphates are sources of highly digestible phosphorus. In this way, they offer a stable, predictable supply of digestible phosphorus that is not affected by other raw materials, production parameters or storage.

Karl De Bruyne is area manager for Tessenderlo Chemie, Brussels, Belgium. This paper was submitted through EMFEMA, the European federation of producers of minerals for animal nutrition.

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Summary of apparent digestibility trials in pigs

	Holland	Denmark
Dicalcium phosphate	65	52
Dicalcium phosphate dihydrate, Aliphos	69	59
Dicalcium phosphate dihydrate, ex gelatine	87	--
Monodicalcium phosphate, Aliphos	--	72
Monodicalcium phosphate, others	73	65
Monocalcium phosphate, Aliphos	83	--
Monocalcium phosphate, others	76	64
Defluorinated phosphate (tricalcium phosphate + Ca Na P)	80	50

	Belgium
Dicalcium phosphate	63
Dicalcium phosphate dihydrate, Aliphos	73
Dicalcium phosphate dihydrate, ex gelatine	--
Monodicalcium phosphate, Aliphos	--
Monodicalcium phosphate, others	--
Monocalcium phosphate, Aliphos	92
Monocalcium phosphate, others	--
Defluorinated phosphate (tricalcium phosphate + Ca Na P)	--

Holland: Grimbergen 1985, Mulder & Jongbloed 1985, Vandertol 1988, Vanderpeet
Denmark: Foulum 1994
Belgium: Eeckhout 1996

Apparent digestibility with poultry; Holland and Belgium

Source	A	B	Average	Relative
Monocalcium phosphate, Aliphos	84	85	85	100
Monocalcium phosphate, others	76	-	76	90
Dicalcium phosphate dihydrate, Aliphos	77	83	80	94
Dicalcium phosphate dihydrate, bone grade	80	80	80	94
Dicalcium phosphate	53	70	62	73
Monodicalcium phosphate, others	79	-	79	94
Defluorinated phosphate	55	-	55	65

A: Simons and Van de Klis 1990-1994
B: Huyghebaert and DeGroote, 1996

Apparent digestibility of different types of
feed phosphates, a summary

Source digestibility	Pigs Phosphorus digestibility	Retative	Poultry Phosphorus
Dicalcium phosphate dihydrate, Aliphos	70	78	80
Dicalcium phosphate, Aliphos	65	72	70
Monocalcium phosphate, Aliphos	90	100	85
Monocalcium phosphate, others	80	89	76
Monodicalcium phosphate, Aliphos	80	89	83
Monodicalcium phosphate, others	75	83	75
Defluorinated phosphate	50-75	55-83	55

Source	Relative
Dicalcium phosphate dihydrate, Aliphos	95
Dicalcium phosphate, Aliphos	82
Monocalcium phosphate, Aliphos	100
Monocalcium phosphate, others	89
Monodicalcium phosphate, Aliphos	98
Monodicalcium phosphate, others	88
Defluorinated phosphate	65

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1. Document ID: US 6291221 B1

L2: Entry 1 of 10 File: USPT Sep 18, 2001

US-PAT-NO: 6291221
DOCUMENT-IDENTIFIER: US 6291221 B1

TITLE: Heat tolerant phytases

DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
van Loon; Adolphus	Rheinfelden			CHX
Mitchell; David	Aesch			CHX

US-CL-CURRENT: 435/196; 435/252.3, 435/254.11, 435/320.1, 435/325, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

2. Document ID: US 6183740 B1

L2: Entry 2 of 10 File: USPT Feb 6, 2001

US-PAT-NO: 6183740
DOCUMENT-IDENTIFIER: US 6183740 B1

TITLE: Recombinant bacterial phytases and uses thereof

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Short; Jay M.	Rancho Santa Fe	CA		
Kretz; Keith A.	San Marcos	CA		

US-CL-CURRENT: 424/94.6; 435/196, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

3. Document ID: US 6153418 A

L2: Entry 3 of 10 File: USPT Nov 28, 2000

US-PAT-NO: 6153418
DOCUMENT-IDENTIFIER: US 6153418 A

TITLE: Consensus phytases

DATE-ISSUED: November 28, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lehmann; Martin	Inzlingen			DEX

US-CL-CURRENT: 435/195; 424/94.1, 424/94.6, 435/196

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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4. Document ID: US 5985605 A

L2: Entry 4 of 10 File: USPT Nov 16, 1999

US-PAT-NO: 5985605
DOCUMENT-IDENTIFIER: US 5985605 A

TITLE: DNA sequences encoding phytases of ruminal microorganisms

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cheng; Kuo Joan	Lethbridge			CAX
Selinger; Leonard Brent	Lethbridge			CAX
Yanke; Lindsey Jay	Lethbridge			CAX
Bae; Hee Dong	Seoul			KRX
Zhou; Luming	Salt Lake City	UT		
Forsberg; Cecil Wallace	Guelph			CAX

US-CL-CURRENT: 800/278; 435/196, 435/252.3, 435/252.31, 435/252.33, 435/254.11, 435/254.23, 435/325, 435/419, 536/23.2, 536/23.7, 800/287, 800/288, 800/294, 800/306

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw. Desc | Image

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5. Document ID: US 5939303 A

L2: Entry 5 of 10 File: USPT Aug 17, 1999

US-PAT-NO: 5939303
DOCUMENT-IDENTIFIER: US 5939303 A

TITLE: Phytases of ruminal microorganisms

DATE-ISSUED: August 17, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cheng; Kuo Joan	Lethbridge			CAX
Selinger; Leonard Brent	Lethbridge			CAX
Yanke; Lindsey Jay	Lethbridge			CAX
Bae; Hee Dong	Seoul			KRX
Zhou; Luming	Salt Lake City	UT		
Forsberg; Cecil Wallace	Guelph			CAX

US-CL-CURRENT: 435/196; 424/94.6, 426/61, 426/635, 435/183, 435/195

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

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☐ 6. Document ID: EP 897985 A2

L2: Entry 6 of 10   File: EPAB   Feb 24, 1999

PUB-NO: EP000897985A2
DOCUMENT-IDENTIFIER: EP 897985 A2
TITLE: Consensus phytases

PUBN-DATE: February 24, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
LEHMANN, MARTIN	DE

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 9/00; A23L 1/03; A23K 1/165; A61K 38/46
EUR-CL (EPC): C12N009/16

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

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☐ 7. Document ID: EP 897010 A2

L2: Entry 7 of 10   File: EPAB   Feb 17, 1999

PUB-NO: EP000897010A2
DOCUMENT-IDENTIFIER: EP 897010 A2
TITLE: Modified phytases

PUBN-DATE: February 17, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
KOSTREWA, DIRK	DE
PASAMONTES, LUIS	CH
TOMSCHY, ANDREA	DE
VAN, LOON ADOLPHUS	CH
VOGEL, KURT	CH
WYSS, MARKUS	CH

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 1/15; C12N 1/19; A23K 1/165

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

8. Document ID: WO 9805785 A1

L2: Entry 8 of 10   File: EPAB   Feb 12, 1998

PUB-NO: WO009805785A1
DOCUMENT-IDENTIFIER: WO 9805785 A1
TITLE: PLANT PHYTASES AND BIOTECHNOLOGICAL APPLICATIONS

PUBN-DATE: February 12, 1998

INVENTOR-INFORMATION:

NAME	COUNTRY
MAUGENEST, SEBASTIEN	FR
LESCURE, ANNE-MARIE	FR
PEREZ, PASCUAL	FR

INT-CL (IPC): C12N 15/55; C12N 15/82; A01H 5/00; A23K 1/165; C08B 30/00; C12P 19/04
EUR-CL (EPC): C12N009/16; C12N015/82, C12N015/82

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

9. Document ID: WO 9748812 A2

L2: Entry 9 of 10   File: EPAB   Dec 24, 1997

PUB-NO: WO009748812A2
DOCUMENT-IDENTIFIER: WO 9748812 A2
TITLE: DNA SEQUENCES ENCODING PHYTASES OF RUMINAL MICROORGANISMS

PUBN-DATE: December 24, 1997

INVENTOR-INFORMATION:

NAME	COUNTRY
CHENG, KUO JOAN	CA
SELINGER, LEONARD BRENT	CA
YANKE, LINDSEY JAY	CA
BAE, HEE DONG	KR
ZHOU, LUMING	US
FORSBERG, CECIL WALLACE	CA

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 1/19; C12N 1/20; C12N 1/21; A01H 5/00; A23K 1/00; A23K 1/165; C12Q 1/44; C12Q 1/68; C12N 15/82
EUR-CL (EPC): C12N009/16; A23K001/165, C12N015/82 , C12N015/82

Full | Title | Citation | Front | Review | Classification | Date | Reference   KWIC | Draw Desc | Image

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10. Document ID: AU 200020928 A, WO 200043503 A1

L2: Entry 10 of 10   File: DWPI   Aug 7, 2000

DERWENT-ACC-NO: 2000-491161
DERWENT-WEEK: 200055

TITLE: Novel phytases with improved properties such as temperature stability, pH stability and substrate specificity, for use in pharmaceuticals and compound foods and feeds

INVENTOR: LEHMANN, M

PRIORITY-DATA: 1999DK-0001340 (September 21, 1999), 1999DK-0000092 (January 22, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200020928 A	August 7, 2000		000	C12N009/16
WO 200043503 A1	July 27, 2000	E	238	C12N009/16

INT-CL (IPC): C12N 9/16

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